experiments, a total of 199 junctional SR and 191 free SR proteins were identified from 16,344 MSMS spectra with protein false discovery rate (FDR) at 0.8% and peptide FDR at 0.0%. SERCA2a was the most abundant protein in both fractions, as expected, and virtually all of the known cardiac SR proteins were also identified. In jSR, the major 4 components of the Ca^{2+}-release complex were found exclusively, including calsequestrin-2, ryanodine receptor 2 and 3, triadin, and junctin. Another 57 proteins were also identified as specific to this subcompartment. In fSR, 53 proteins were exclusively identified. Relative enrichments in one of the SR subcompartments were found for an additional 86 proteins, and 52 additional proteins were identified in the cardiac SR, without obvious subcompartment origin. SR proteins that showed less specificity were mitochondrial proteins (more enriched in the lower-density jSR), ER chaperones (roughly evenly distributed), lipid-metabolizing enzymes, and filamentous proteins. Proteomic analyses of classical cardiac SR subfractions extends our understanding of the cardiac secretory compartments, and serves as foundation for future exploration and understanding of cardiac cell biology.

1044-Plat
Cav1.1 Controls ATP Release in Adult Muscle Fibers
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In adult muscle fibers, Cav1.1 acts as voltage sensor for both excitation-contraction coupling and the activation of a signaling cascade that regulates gene expression. We have shown that ATP is released trough pannexin-1 channels after electrical stimulation at 20 Hz, having a key role in the induction of transcriptional changes related to fast-to-slow muscle fiber phenotype transition. Myotubes lacking the Cav1.1-α1 subunit displayed almost no ATP release after electrical stimulation. The same was observed in adult fibers treated with the Cav1.1 antagonist nifedipine (25μM), showing that Cav1.1 has a central role controlling ATP release.
We examined the activation of this signaling cascade in muscle fibers where a knock-down of the α1s subunit of Cav1.1 was obtained by a U7-exon skipping strategy using adenovirus-associated viral vectors (AAV-U7delα1s).
Four months after AAV-U7delα1s injection we observed a significant reduction of Cav1.1 protein as well as atrophy and fibrosis of the treated muscles. Indo-1 Ca^{2+} transients and Ca^{2+} current in voltage-clamped fibers isolated from FDB treated muscles showed that the peak Ca^{2+} transient elicited by short depolarizing pulses was reduced by 35% whereas the maximal conductance of the Ca^{2+} channels was reduced by 30%. We also found increased basal ATP release with spontaneous release events. AAV-U7delα1s treated fibers showed higher mRNA levels of the slow isoform of Troponin I and lower mRNA levels of the fast isoform of Troponin I compared with non-treated muscles. The transcriptional changes observed in these two genes after electrical stimulation were absent in AAV-U7delα1s treated fibers.
These results suggest that Cav1.1 controls ATP release trough Pannexin-1 channels, activating it after low stimulation frequencies and blocking ATP release during resting. The loss of this control perturbs the normal transcriptional response to electrical activity of adult muscle fibers.
ACT1111, FONDECYT 1110467, FONDAP 15010006

Platform: Enzymes

1045-Plat
Long Duration, Single-Molecule Monitoring of Lysozyme Kinetics
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Tethering a single molecule of T4 lysoyzme to a carbon nanotube field effect transistor resulted in a novel technique for monitoring single-molecule enzymatic activity. Continuous electronic monitoring readily resolved lysozyme as a processive enzyme, since substrate release was clearly distinguished from serial hydrolysis of glycosidic bonds. On average, we observed hydrolysis of 100 consecutive bonds at 15 Hz rates when lysozyme processed natural peptidoglycan. Furthermore, long duration measurements allowed statistically-meaningful analysis of thousands of chemical events by the same molecule, uncovering seven independent timescales governing lysozyme's activity, including minute-by-minute dynamic disorder. Stability of the lysozyme-transistor method allowed these timescales to be studied as a function of environmental conditions such as substrate, pH, or temperature, in order to produce a detailed map of factors that affect single molecule processivity. For example, variations in pH do not change any of the rate constants of lysozyme's motions, but rather decrease enzyme activity by increasing the proportion of time stuck in an inactive, closed conformation. As another example, we found that peptidoglycan cross-links are directly responsible for a 50% drop in effective activity. Using a synthetic, linear substrate without cross-links, we observed long, uninterrupted processing runs to the end of individual substrate molecules. By comparison, lysozyme spends approximately half its time traversing the cross-links present in wild-type substrate. Rather than releasing substrate at a cross-link, however, lysozyme instead changes to an alternate, non-processive motion with which it can sidestep the cross-link. The combination of processive and non-processive motions allow lysozyme to remain effective and zigzag through wild-type substrate.

1046-Plat
Single Molecule Kinetics of cAMP-Dependent Protein Kinase Monitored Continuously by a Carbon Nanotube Transistor
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cAMP-dependent Protein Kinase (PKA) is a protein that plays a critical role in cell signaling by means of protein phosphorylation. In this work, the kinetics of PKA activity were monitored with the aid of a single-walled carbon nanotube transistor. Molecule-by-molecule processivity was directly recorded using an individual PKA catalytic subunit attached to this sensitive transducer. The binding of adenosine triphosphate (ATP) and/or kemptide, a peptide substrate, both drove conformational changes that could be electrically monitored with sub-millisecond resolution over durations of 10 minutes or more. In environments containing either binding partner, binding and dissociation rates and rate variability were determined from many thousands of individual events. For ATP, the on-rate varies over a range of 20-1000 Hz due to dynamic disorder of the apoenzyme. In fact, single molecule monitoring allowed us to directly record periods of fast and slow binding. In the presence of both ATP and kemptide, more complicated signals resulted from PKA alternating between its apo, binary, and catalytically-active ternary complex. This three-state system was extensively monitored to determine its transition probability matrix and rates. Within our resolution of 0.1ms, the apoenzyme preferentially forms the ternary-complex, with only 27% of binding events pausing at the intermediate, binary complex. Our single molecule measurements observed a varying catalytic turnover rate of 5-100 Hz over 10 sec intervals with a time averaged rate of 30 Hz, consistent with the 20 Hz rate from ensemble measurements.

1047-Plat
NucS DNA Flap Interaction Mechanism Kinetics Revealed by Single Molecule Imaging
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DNA reparation enzymes are essential to preserve genome integrity. The understanding of the dynamics of the regulation pathways of their activity is thus crucial. *Pyrococcus abyssi* NucS is a recently discovered archaeal endonuclease acting on branched DNA. The biochemical characterization of *Pab*NucS has shown that this protein binds ssDNA at nanomolar concentrations and has a rare bidirectional activity both on 5' and 3' free ssDNA extremities. Although ensemble measurements indicate that free ssDNA extremity is necessary for NucS activity, the detailed mechanisms that regulate NucS activity remain unclear. In particular, it remains unknown whether NucS loads at the extremity of the flap and then diffuses to the junction, or whether protein directly binds to the ss/dsDNA junction.
To probe the dynamics of *Pab*NucS ssDNA interactions, we use single-molecule tracking of fluorescently-labeled *Pab*NucS acting on a DNA substrate tethered to the surface. We report a bidirectional and oriented binding on 3' and 5' flaps at the single molecule level. We measured binding/unbinding kinetics with unprecedent accuracy and revealed that NucS/DNA flap unbinding follows a diffusion independent multistep mechanism that depends on the NucS/ssDNA energy landscape.
Consistently with the structural information of the high affinity ssDNA binding site that displays a hydrophobic core surrounded by charged residues, we propose a two-step unbinding model that allows a stochastic unbinding of the flap and characterize two forces contributing to the interaction: (i) an electrostatic force that controls binding and (ii) a non electrostatic (hydrophobic) force that is dominant at high salt concentration and maintains NucS-DNA complex stability.

Our results constitute a notable contribution to the characterization of the ssDNA-NucS interactions and more generally to the nucleotide excision repair mechanism. The method we developed furthermore constitutes a powerful way to probe DNA/protein intramolecular kinetics.

1048-Plat
Construct a Catalyst using the Beta-Peptide Bundle
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''What I cannot create, I do not understand.'' Richard Feynman, 1988.
Through the construction of an efficient catalyst using a non-natural material, this research project aims to deepen our understanding of the structural and energetic features that control catalysis. We expect our findings to inform the principles for the design and engineering of both proteinaceous enzymes and artificial catalysts.
We set out to use beta-peptides - a unique yet virtually unexplored middle ground between proteins and small molecules - to devise catalysts for the formation or hydrolysis of glycosidic bonds. Beta-peptides are smaller and thus synthetically more tractable than natural proteins; nevertheless, they can still adopt higher order structures inaccessible to small molecules. For example, the Schepartz Laboratory reported previously that the EYYK β-peptide self-assembles into an octameric bundle that displays conformational flexibility and thermodynamic properties similar to natural protein. We therefore build our first generation design around the EYYK scaffold.
Each beta-peptide bundle will be characterize by three biophysical techniques. In addition to structural information obtained by X-ray crystallography, two novel assays that evaluate the beta-peptide bundles based on their substrate binding affinity and reaction turnover rate will be carried out. Together these information provide details of the physical and chemical properties of the beta-peptides to guide the iterative design - synthesis - characterization process. The latest results of our first generation beta-peptide bundle, EYBK, will be presented. The utility of two newly developed assays for monitoring binding and turnover will also be discussed in the context of informing catalyst design.

1049-Plat
An Artificial Safranine Enzyme which Activates Chemotherapeutic Prodrugs
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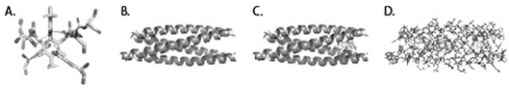
Protein design has opened new boundaries in the past decade, particularly in the area of designed enzymes capable of catalyzing reactions not yet observed in nature. We report the design and synthesis of an artificial enzyme capable of activating the class of nitroaromatic anticancer prodrugs typified by CB1954. The enzyme utilizes a synthetic flavin analogue cofactor, p-methoxysafranine, which has a reduction potential preoptimized for efficient nitroreduction. Progress toward optimizing catalytic activity and determination of the three-dimensional structure of this novel enzyme will also be reported.



1050-Plat
Redox Enzymology of Shewanella Oneidensis Cytochrome C Nitrite Reductase
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Shewanella oneidensis cytochrome c nitrite reductase (soNrfA), a dimeric enzyme that houses five c-type hemes per protomer, carries out the six-electron reduction of nitrite and the two-electron reduction of hydroxylamine. Protein film voltammetry (PFV) has previously been used to study the cytochrome c nitrite reductase from Escherichia coli (ecNrfA) adsorbed to a graphite electrode, revealing catalytic reduction of both nitrite and hydroxylamine substrates by ecNrfA that is characterized by 'boosts' and attenuations in activity depending on the applied potential. Here, we use PFV to investigate the catalytic properties of soNrfA during both nitrite and hydroxylamine turnover and compare those properties to ecNrfA. Distinct differences in both the electrochemical and kinetic characteristics of soNrfA are observed, e.g., all detected electron transfer steps are one-electron in nature, contrary to what has been observed in ecNrfA. Additionally, we find evidence of substrate inhibition during nitrite turnover and negative cooperativity during hydroxylamine turnover, neither of which have previously been observed in any cytochrome c nitrite reductase. Collectively these data provide evidence that during catalysis, potential pathways of communication exist between the individual soNrfA monomers comprising the native homodimer.

1051-Plat
Vibrational Stark Effects in the Active Site of Ketosteroid Isomerase Point to Large Electric Fields Driving Chemical Catalysis
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Enzymes are extraordinary catalysts that actuate nearly all biomolecular processes with speed and specificity. Nevertheless, the physical origins of enzymes' catalytic power remain elusive despite investigations of many enzymes' mechanisms over the last half-century. Ketosteroid isomerase (KSI) is a small and proficient enzyme - accelerating an isomerization reaction ~1 trillion-fold over its intrinsic rate in water - that has been employed as a test system to examine the catalytic strategies at Nature's disposal. Electrostatic interactions are broadly purported to play an essential role in catalysis, but this proposal has yet to be experimentally tested in a quantitative fashion. Toward this end, vibrational Stark effect spectroscopy provides a toolkit to measure electric fields in biomolecular environments. By measuring the vibrational Stark effect of an intrinsic probe that mimics the reactive species of KSI's catalytic cycle, we found that KSI's active site focuses an extremely large electric field onto the scissile bond, potentially enabling its speedy chemical conversion through electrostatic interactions. Moreover, we observed a strong correlation between active site electric field and catalytic power across several KSI mutants. These studies are building toward a highly reductionist picture of KSI's catalysis and possibly enzyme catalysis in general.

1052-Plat
Pseudosubstrate Regulation of the Type II P21-Activated Kinases (PAKs)
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The type II p21-activated kinases (PAKs) are key effectors of RHO-family GTPases involved in cell motility, survival and proliferation. There are two sub-groups of PAK kinase, denoted type I (PAK1, PAK2 and PAK3) and type II (PAK4, PAK5 and PAK6). PAK4 is the best-studied type II PAK family member, is widely expressed, and is essential for viability in mice. PAK4 is required for oncogenic or metastatic phenotypes of many cancer cell lines, can promote tumorigenesis and breast cancer cell migration, and is the only PAK that is transforming when overexpressed. Furthermore, a specific PAK4 inhibitor (PF-3758309) has shown efficacy in mouse models of cancer. Using a structure-guided approach, we discovered that type II PAKs are regulated by an N-terminal autoinhibitory pseudosubstrate motif centered on a critical proline residue, and that this regulation occurs independently of activation loop phosphorylation. We determined 6 X-ray crystal structures of either full-length PAK4 or its catalytic domain, that demonstrate the molecular basis for pseudosubstrate binding to the active state with phosphorylated activation loop. We show that full-length PAK4 is constitutively autoinhibited, but mutation of the pseudosubstrate releases this inhibition and causes increased phosphorylation of BAD and cellular morphological changes. We also find that PAK6 is regulated by the pseudosubstrate region, indicating a common type II PAK autoregulatory mechanism. Finally, we find Src SH3, but not β-PIX SH3 can activate PAK4. This clear switch-like 'on-off' regulation mechanism for the type II PAKs is distinct from regulation mechanisms previously observed for the type I PAKs. We therefore provide new understanding for type II PAK regulation.

Symposium: Regulation of Protein Synthesis

1053-Symp
Optimal Strategy for Rapid Proteome Re-Arrangements in Bacterial Populations
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When a bacterial population is subjected to environmental change, its proteome must be re-arranged for resumption of rapid growth. Upon recurrent environmental changes, rapid proteome responses are required for high average growth rate and fitness value, suggesting high selection pressure for rapid proteome re-arrangements in response to environmental changes. What, then, is the best gene expression strategy for rapid proteome change among bacteria?
Here, we demonstrate that the optimal solution to the adaptation problem is to direct all gene expression to synthesis of the proteins in the currently rate limiting set of pathways. The result is obtained by reformulating proteome dynamics in terms of flow-coupled networks in which the rate limiting protein components determine the growth rate. This approach provides a universal frame work for the description of the whole proteome and its adaptation to